Box Seq.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: USB98ASIDM

In the patent application of

MIDOUX, PATRICK *et al.*

Serial No. 09/857,448

Filed: June 4, 2001

For: NEW OLIGOMERIC CONJUGATES LIABLE TO TRANSFER BIOLOGICAL MOLECULES INTO CELLS

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;

2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same; and

3. all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

YOUNG & THOMPSON

By _____

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October 9, 2001

#4

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Kazuo KONUMA

Serial No. 09/506,003 GROUP 2875

Filed February 17, 2000 Examiner S. O'Shea

ELECTRON EMISSION DEVICE

AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Responsive to the Official Action of July 10, 2001, please amend the above-identified application as follows:

IN THE TITLE:

Change the title throughout, declaration excepted, to: --ELECTRON EMISSION DEVICE WITH PICTURE ELEMENT ARRAY--.

IN THE ABSTRACT:

Rewrite the Abstract of the Disclosure as on the attached separate sheet.

IN THE SPECIFICATION:

Page 2, replace the paragraph beginning on line 17 as follows:

--On the other hand, the distance between the gate electrode 3 and the emitter 5 is 1 μm (10^{-3}[mm]), and the voltage

Add the following new claim:

--31. (new) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter are converged toward said anode electrode side, and

wherein said gate electrode is formed as a nearly rectangle and a plurality of said openings are formed at the center and corners of said gate electrode, in the opening located at the center, the emitter extending longitudinally along said gate electrode at the center of the opening, in the openings located at the corners, the emitters extending shifted outside from the center of said openings.--

Please charge the fee of $168.00 for the addition of two independent claims, added herewith, to Deposit Account No. 25-0120.

is 100 V. So, the electric field between the both electrodes 3 and 5, gate and emitter, is given by:

$100/10^{-3}$[V/mm]=100[kV/mm].--

Page 23, replace the last paragraph bridging pages 23 and 24, as follows:

--Next, the target region that electrons plunge into the anode electrode 6 is considered. At first, when the circular hole lens functions as a divergence lens, the target region becomes wider than the emitter region at the bottom of gate hole 4 and is never narrower than that. On the other hand, when the circular hole lens functions as a convergence lens, the target region becomes wider or narrower than or the same as the emitter region, as the case may be. Whether the target region becomes wider or narrower cannot be determined by only the definition of electric field made in the consideration of equipotential surface. For example, in both cases that a voltage of 1 kV is applied while departing 1 mm the anode electrode 6 from the emitter 5, and that a voltage of 10 kV is applied while departing 10 mm the anode electrode 6 from the emitter 5, electric field of 1 kV/mm can be obtained. However, for electron passing the convergence lens system, the target region can be narrower due to before or just after the over-focusing when the distance between the cathode and anode electrodes is 1 mm, or the target region

can be wider due to the sufficient over-focusing when the distance between the cathode and anode electrodes is 10 mm.—

Page 25, replace the first full paragraph as follows:

--In FIG. 9, shown are only electron 10 emitted from the outside of cathode electrode 5 deposited at the bottom of gate hole 4, and electron 9 emitted form the center. The electric field between gate electrode 3 and cathode electrode 5 is set to be smaller than the electric field between anode electrode 6 and gate electrode 3. Thereby, the electric field formed inside the gate hole 4 and in its vicinity yields a lens effect to enable electron emitted to the anode electrode 6 to be converged. Therefore, electrons plunge into the anode electrode 6 while being over-focused. This over-focusing state does not always occur, and occurs only when the convergence effect is significant. When the state is approximate to equal electric field, electrons plunge into the anode electrode 6 while being under-focused or just-focused.--

Page 30, replace the second paragraph as follows:

--Based on the analysis in FIG. 18, this embodiment is further explained. FIG. 19 shows an example of electron emission device to offer the widening characteristics of the acceptable emission widening range 19 in FIG. 18, where the pixel 13 is viewed from the electron emission position. In FIG. 19, three

gate holes (openings) 4 are arrayed linearly in gate electrode 3, and the emitters 5 formed in the gate holes 4 are aligned. Namely, in the bottom of each of the three tubular gate holes 4 with a diameter of e.g. 10 μm formed in gate electrode 3, emitter 5 shaped like a rectangle is coated. Under the gate electrode 3, insulation film (not shown) is formed.--

IN THE CLAIMS:

Amend claim 1 as follows:

--1. (amended) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter are converged toward said anode electrode side,

said convergence electric field has equipotential surface whose inclination increases from the anode electrode to the gate electrode, and

a relationship represented by expression below is satisfied:

$$\{t(gk)/t(ak)\}\cdot Va<Vg<[\{t(g)+t(gk)\}/t(ak)]\cdot Va$$

where distance between the surface of said emitter and the back surface of said gate electrode is t(gk), distance between the back surface of said anode electrode and the surface of said emitter is t(ak), potential of said anode electrode is Va, potential of said gate electrode is Vg, and thickness of said gate electrode is t(g).--

Amend claim 2 as follows:

--2. (amended) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter are converged toward said anode electrode side,

wherein said convergence electric field is formed by setting so that gate/emitter electric field given between said gate electrode and said emitter is smaller than gate/anode electric field given between said gate electrode and said anode electrode, and

wherein said gate electrode is formed as a nearly rectangle and a plurality of said openings are formed at the center and corners of said gate electrode, in the opening located at the center, the emitter extending longitudinally along said gate electrode at the center of the opening, in the openings located at the corners, the emitters extending shifted outside from the center of said openings.--

Cancel claim 3.

Cancel claim 15.

Amend claim 17 as follows:

--17. (amended) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field by which electrons to be emitted from said emitter is converged toward said anode electrode side is formed,

wherein said convergence electric field is formed by setting so that gate/emitter electric field given between said

gate electrode and said emitter is smaller than gate/anode electric field given between said gate electrode and said anode electrode,

wherein said opening is formed as a nearly rectangle, and said emitter is formed like a strip along the longitudinal direction of said opening, and

wherein three picture elements, each of which is composed of said emitter and said gate electrode, are arrayed adjacent to each other,

the emitter of the first picture element located at the center of said three picture elements extends longitudinally at the center of said nearly rectangular opening,

the emitter of the second picture element located at one end of said three picture elements extends longitudinally on said first picture element side of said nearly rectangular opening, and

the emitter of the third picture element located at another end of said three picture elements extends longitudinally on said first picture element side of said nearly rectangular opening.--

Cancel claim 19.

Add the following new claim:

--31. (new) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter are converged toward said anode electrode side, and

wherein said gate electrode is formed as a nearly rectangle and a plurality of said openings are formed at the center and corners of said gate electrode, in the opening located at the center, the emitter extending longitudinally along said gate electrode at the center of the opening, in the openings located at the corners, the emitters extending shifted outside from the center of said openings.--

Please charge the fee of $168.00 for the addition of two independent claims, added herewith, to Deposit Account No. 25-0120.

R E M A R K S

This application has been amended so as to place it in condition for allowance at the time of the next Official Action.

The Official Action objects to the specification as failing to comply with 35 USC §112, first paragraph. Underlying this objection are identified passages in the narrative portion of the specification which are considered to be unclear. Applicant has amended the specification as necessary to eliminate the bases for this objection, and reconsideration and withdrawal thereof are therefore respectfully requested.

The Official Action indicates that the title of the invention is not descriptive. Please note that applicant has amended the title by adopting the helpful recommendation included with the Official Action.

The Official Action objects to the abstract for use of the introductory phrase "disclosed is". Applicant includes herewith a replacement abstract which eliminates the objectionable language, and reconsideration and withdrawal of this objection are therefore respectfully requested.

The Official Action objects to claims 1, 2, and 20 based on language in each of the claims considered informal. In connection with claims 1 and 2, applicant has amended the claims to adopt the recommendation helpfully included with the Official Action. Applicant has cancelled claim 20. Reconsideration and

withdrawal of this objection are therefore respectfully requested.

The Official Action objects to claim 3 under 37 CFR §1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant has cancelled claim 3, and reconsideration and withdrawal of this rejection are therefore respectfully requested.

The Official Action rejects claims 1, 3, 4, 6, 8, 10, 12, 14, 16, 18, 22, 25, and 28 under 35 USC §112, first paragraph as failing to meet the enablement requirement thereof. Underlying this rejection is the recitation of an electric field that has equipotential surfaces whose inclinations increase as they approach the emitter. Applicant has amended independent claim 1 to replace the identified language with the recitation that the equipotential surface inclination increases from the anode electrode to the gate electrode, which characteristic is readily disclosed in the specification and illustrated by at least Figure 4. Reconsideration and withdrawal of this rejection are therefore respectfully requested.

The Official Action rejects claim 3 under 35 USC §112, second paragraph, as being indefinite. As discussed above, applicant has cancelled claim 3, and reconsideration and withdrawal of this rejection are therefore respectfully requested.

The Official Action rejects claim 20 under 35 USC §102(e) as being anticipated by KANE et al. 5,252,833. Applicant has cancelled claim 20, and reconsideration and withdrawal of this rejection are therefore respectfully requested.

The Official Action rejects claim 2 under 35 USC §103(a) as being unpatentable over SPINDT et al. 5,528,103. Paragraph 21 of the Official Action explicitly notes, however, that claim 19 is among those considered allowable but for their dependence from rejected base claims. Accordingly, applicant has amended independent claim 2 to recite the features of original claim 19. Such amendment should necessarily serve to overcome the present rejection and place claim 2 into condition for immediate allowance. Reconsideration and withdrawal of this rejection are therefore respectfully requested.

The Official Action rejects claims 5 and 7 under 35 USC §103(a) as being unpatentable over SPINDT et al. and further in view of YUITO et al. 4,008,412. Each of the rejected claims depends directly from claim 2 which, as discussed above, has been amended to be in condition for allowance. Claims 5 and 7 should therefore also be in condition for allowance at least by virtue of such dependence. Reconsideration and withdrawal of the present rejection are therefore respectfully requested.

The Official Action rejects claim 9 under 35 USC §103(a) as being unpatentable over SPINDT et al. and further in view of SHELTON 3,783,325. Claim 9 depends from claim 2, which

has been amended so as to be in condition for allowance. Claim 9 should be allowable for at least this reason, and reconsideration and withdrawal of the present rejection are therefore respectfully requested.

The Official Action rejects claim 11 under 35 USC §103(a) as being unpatentable over SPINDT et al., and further in view of HSU et al. 6,084,145. Claim 11 depends from claim 2, which is believed to be in condition for allowance, and reconsideration and withdrawal of the present rejection are therefore respectfully requested.

The Official Action rejects claims 13, 15, 23, 26, and 29 under 35 USC §103(a) as being unpatentable over SPINDT et al. and further in view of BETSUI et al. 5,489,933. Each of these claims depends directly from claim 2 which, as discussed above, has been amended to be in condition for allowance. The present claims should therefore also be in condition for allowance, at least by virtue of such dependence, and reconsideration and withdrawal of the present rejection are therefore respectfully requested.

The Official Action rejects claim 21 under 35 USC §103(a) as being unpatentable over KANE et al. The Official Action also rejects claims 24, 27, and 30 under 35 USC §103(a) as being unpatentable over KANE et al. and further in view of BETSUI et al. Applicant has cancelled claims 21, 24, 27, and 30, and

reconsideration and withdrawal of the present rejection are therefore respectfully requested.

The Official Action explicitly states that claims 17 and 19 are allowable but for their dependence from rejected base claims. As discussed above in connection with the rejection of claim 2, applicant has amended independent claim 2 to incorporate the features of original claim 19. Applicant has also amended claim 17 into independent form by incorporating the features originally recited in claims 2 and 15, from and through which claim 17 originally depended.

The Official Action explicitly states that claim 1 is allowable but for the language underlying the rejection of such claim under 35 USC §112, first paragraph, discussed above. As applicant has amended claim 1 in order to overcome such rejection, claim 1 should now be in condition for immediate allowance.

The Official Action explicitly states that claims 4, 6, 8, 10, 12, 14, 16, 18, 22, 25 and 28 are allowable but for the rejection of claim 1, from which each of the rejected claims depends. As claim 1 has been amended to overcome their rejection applied against such claim, all claims depending from claim 1 should also be in condition for immediate allowance.

In addition to the amendments described above, applicant has added new claim 31. Claim 31 is an independent claim similar to amended claim 2. Claim 31, however, while

reciting the feature originally recited in allowable claim 19, does not recite the feature of original claim 2 relating to the relationship between the electric field between the gate and emitter, and the electric field between the gate and the anode. As such claim recites features which are neither anticipated by nor obvious in light of the prior art, applicant respectfully suggests that new claim 31 is also in condition for immediate allowance.

Applicant has also amended two passages on page 2 of the specification. The first merely replaces the "E" with the alternative convention for exponential notation. In the second passage, the proper units are added to the left side of the equation so that the equation balances.

In light of the amendments described above and the arguments offered in support thereof, applicant believes that the present application is in condition for allowance and an early indication of the same is respectfully requested.

If the Examiner has any questions or requires further clarification of any of the above points, the Examiner may contact the undersigned attorney so that this application may continue to be expeditiously advanced.

Attached hereto is a marked-up version of the changes made to the specification, claims, and abstract by the current

amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,

YOUNG & THOMPSON

By _____

Eric Jensen
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October 9, 2001

Abstract of the Disclosure

An electron emission device includes: a substrate; a gate electrode that has an opening and is disposed on the substrate; an emitter that is formed in the opening; and an anode electrode that is disposed at a predetermined interval from the emitter. In this device, a convergence electric field by which electrons to be emitted from the emitter is converged toward the anode electrode side is formed, the convergence electric field has equipotential surface whose inclination increases according as it approaches the emitter, and a relationship represented by expression below is satisfied:

$$\{t(gk)/t(ak)\}\cdot Va<Vg<[\{t(g)+t(gk)\}/t(ak)]\cdot Va.$$

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

The title has been amended as follows:

--ELECTRON EMISSION DEVICE WITH PICTURE ELEMENT ARRAY-.

IN THE SPECIFICATION:

Page 2, the paragraph beginning on line 17 has been replaced as follows:

--On the other hand, the distance between the gate electrode 3 and the emitter 5 is 1 μm [($1E^{-3}$[mm])] (10^{-3}[mm]), and the voltage is 100 V. So, the electric field between the both electrodes 3 and 5, gate and emitter, is given by:

[$100/1E^{-3}$=100 [kV/mm].]

$100/10^{-3}$[V/mm]=100[kV/mm].--

Page 23, the last paragraph bridging pages 23 and 24, is amended as follows:

--Next, the target region that electrons plunge into the anode electrode 6 is considered. At first, when the circular hole lens functions as a divergence lens, the target region becomes wider than the emitter region at the bottom of gate hole 4 and is never narrower than that. On the other hand, when the circular hole lens functions as a convergence lens, the target region becomes wider or narrower than or the same as the emitter region, as the case may be. Whether the target region becomes

wider or narrower cannot be determined by only the definition of electric field made in the consideration of equipotential surface. For example, in both cases that a voltage of 1 kV is applied while departing 1 mm the anode electrode 6 from the [anode electrode] emitter 5, and that a voltage of 10 kV is applied while departing 10 mm the anode electrode 6 from the [anode electrode] emitter 5, electric field of 1 kV/mm can be obtained. However, for electron passing the convergence lens system, the target region can be narrower due to before or just after the over-focusing when the distance between the cathode and anode electrodes is 1 mm, or the target region can be wider due to the sufficient over-focusing when the distance between the cathode and anode electrodes is 10 mm.-

Page 25, the first full paragraph is amended as follows:

--In FIG. 9, shown are only electron [12] 10 emitted from the outside of cathode electrode 5 deposited at the bottom of gate hole 4, and electron 9 emitted form the center. The electric field between gate electrode 3 and cathode electrode 5 is set to be smaller than the electric field between anode electrode 6 and gate electrode 3. Thereby, the electric field formed inside the gate hole 4 and in its vicinity yields a lens effect to enable electron emitted to the anode electrode 6 to be converged. Therefore, electrons plunge into the anode electrode

6 while being over-focused. This over-focusing state does not always occur, and occurs only when the convergence effect is significant. When the state is approximate to equal electric field, electrons plunge into the anode electrode 6 while being under-focused or just-focused.-

Page 30, the second paragraph is amended as follows:

--Based on the analysis in FIG. 18, this embodiment is further explained. FIG. 19 shows an example of electron emission device to offer the widening characteristics of the acceptable emission widening range 19 in FIG. 18, where the [emitter 5] pixel 13 is viewed from the electron emission position. In FIG. 19, three gate holes (openings) 4 are arrayed linearly in gate electrode 3, and the emitters 5 formed in the gate holes 4 are aligned. Namely, in the bottom of each of the three tubular gate holes 4 with a diameter of e.g. 10 μm formed in gate electrode 3, emitter 5 shaped like a rectangle is coated. Under the gate electrode 3, insulation film (not shown) is formed.--

IN THE CLAIMS:

Claim 1 is amended as follows:

--1. (amended) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter [is] are converged toward said anode electrode side [is formed],

said convergence electric field has equipotential surface whose inclination increases [according as it approaches said emitter] from the anode electrode to the gate electrode, and

a relationship represented by expression below is satisfied:

$$\{t(gk)/t(ak)\}\cdot Va<Vg<[\{t(g)+t(gk)\}/t(ak)]\cdot Va$$

where distance between the surface of said emitter and the back surface of said gate electrode is t(gk), distance between the back surface of said anode electrode and the surface of said emitter is t(ak), potential of said anode electrode is Va, potential of said gate electrode is Vg, and thickness of said gate electrode is t(g).--

Claim 2 is amended as follows:

--2. (amended) An electron emission device, comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field is formed by which electrons to be emitted from said emitter [is] are converged toward said anode electrode side [is formed], [and]

wherein said convergence electric field is formed by setting so that gate/emitter electric field given between said gate electrode and said emitter is smaller than gate/anode electric field given between said gate electrode and said anode electrode, and

wherein said gate electrode is formed as a nearly rectangle and a plurality of said openings are formed at the center and corners of said gate electrode, in the opening located at the center, the emitter extending longitudinally along said gate electrode at the center of the opening, in the openings located at the corners, the emitters extending shifted outside from the center of said openings.--

Claim 17 is amended as follows:

--17. (amended) An electron emission device, [according to claim 15,] comprising:

a substrate;

a gate electrode that has an opening and is disposed on said substrate;

an emitter that is formed in said opening; and

an anode electrode that is disposed at a predetermined interval from said emitter;

wherein a convergence electric field by which electrons to be emitted from said emitter is converged toward said anode electrode side is formed,

wherein said convergence electric field is formed by setting so that gate/emitter electric field given between said gate electrode and said emitter is smaller than gate/anode electric field given between said gate electrode and said anode electrode,

wherein said opening is formed as a nearly rectangle, and said emitter is formed like a strip along the longitudinal direction of said opening, and

wherein [:] three picture elements, each of which is composed of said emitter and said gate electrode, are arrayed adjacent to each other,

the emitter of the first picture element located at the center of said three picture elements extends longitudinally at the center of said nearly rectangular opening,

the emitter of the second picture element located at one end of said three picture elements extends longitudinally on

said first picture element side of said nearly rectangular opening, and

the emitter of the third picture element located at another end of said three picture elements extends longitudinally on said first picture element side of said nearly rectangular opening.--

IN THE ABSTRACT:

The Abstract of the Disclosure has been amended as follows:

--[Disclosed is an] An electron emission device [which has] includes: a substrate; a gate electrode that has an opening and is disposed on the substrate; an emitter that is formed in the opening; and an anode electrode that is disposed at a predetermined interval from the emitter. In this device, a convergence electric field by which electrons to be emitted from the emitter is converged toward the anode electrode side is formed, the convergence electric field has equipotential surface whose inclination increases according as it approaches the emitter, and a relationship represented by expression below is satisfied:

$\{t(gk)/t(ak)\}\cdot Va<Vg<[\{t(g)+t(gk)\}/t(ak)]\cdot Va$. --

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Patrick MIDOUX et al.

Serial No. 09/857,448 Group 1645

Filed June 4, 2001

NEW OLIGOMERIC CONJUGATES
LIABLE TO TRANSFER BIOLOGICAL
MOLECULES INTO CELLS

AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Responsive to the Official Action of August 8, 2001, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Replace the paragraph beginning at page 21, line 10 and ending at line 20, with the following rewritten paragraph:

--As example of oligonucleotides, one may cite the following:

GEM 91

phosphorothioate (X = S) oligonucleotide i = 25 (SEQ ID NO: 1)

CTC TCG CAC CCA TCT CTC TCC TTC T

complementary to the AUG initiation site of gag HIV-1 gene

ISIS 1939

phosphorothioate (X = S) oligonucleotide i = 19 (SEQ ID NO: 2)

CCC CCA CCA CTT CCC CTC T

complementary to the 3′ non coding region of ICAM-1 mRNA.--

Replace the paragraph beginning at page 25, line 25, with the following rewritten paragraph:

--As an example of the specific TFO is an oligonucleotide 5′-$A_4GA_4G_6A$-3′ (SEQ ID NO: 6) directed against the polypurine track (PPT) in the NEF-HIV-1 gene.--

Replace the paragraph beginning at page 27, line 1, with the following rewritten paragraph:

--A) Cells are incubated for 4 h at 37°C with 1 µM fluorescein-labelled peptide (*F*-*S*-CGEEDTSEKDEL) (SEQ ID NO:3) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.--

Replace the paragraph beginning at page 27, line 10, with the following rewritten paragraph:

--B) Dendritic cells are incubated for 4 h at 37°C with 1 µM c-myc epitope peptide (SMEQKLISEEDLNFELDEA) (SEQ ID

NO:4) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde in the presence of 0.5 % saponine, washed and then incubated for 1 h with anti c-myc epitope monoclonal antibody (9E10) in PBS containing 10 mg/ml BSA and 0.1% saponin. Cells are washed and further incubated for 1 h in the presence of fluorescein-labelled anti-mouse IgG F(ab)' fragments in PBS containing 10 mg/ml BSA and 0.1% saponin. Cells are washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.--

Replace the paragraph beginning at page 28, line 9, with the following rewritten paragraph:

--Dentritic cells were incubated for 4 h at 37°C with the nonadecapeptide (185-203) from the C-terminal part of the HIV-1 Nef protein containing the nonapeptide (190-198) (AFHHVAREL) (SEQ ID NO:5) in the absence or in the presence of an histidylated oligolysine. Cells are washed and further incubated for 24 h at 37°C in the absence of peptide and histidylated oligolysine. MHC class I presentation of peptide antigen was evaluated by Cr^{51} cytoxic assay by using a CTL clone sensible to the peptide. DCs were labeled with Cr^{51} (target cells : T) and then incubated at 37°C for 4 h in the presence of the CTL clone

(effector cells : E) at E/T ratios ranged from 1 to 100. The supernatants are collected and the radioactivity in the supernatant was recorded. The % of specific Cr^{51} release is calculated according to $(A_{NS} - A_S)/A_{NS}$ X 100 where A_{NS} and A_S are the radioactivity in supernatant dilutions of dentritic cells incubated in the absence and the presence of CTL cells, respectively.--

Replace the paragraph beginning at page 30, line 20, with the following rewritten paragraph:

--Figure 1 shows the activity of GEM-91, an antisense phosphorothioate oligonucleotide (PS-ODN) (CTC TCG CAC CCA TCT CTC TCC TTC T) (SEQ ID NO:1) complementary to the AUG initiation site of gag HIV-1 gene. The effect of histidylated oligolysines was evaluated by using pRET-Luc cells (a rabbit smooth muscle cell line). These cells produce endogenous luciferase under the control of the human phosphoglycerate kinase promoter and the luciferase gene sequence around the AUG codon was replaced by the initiator AUG codon and several downstream codons of gagHIV-1 gene. The results showed that the activity of GEM-91 (IC_{50} > 5 µM) was increased more than 10 times in the presence of 20 µm HoK2 (IC_{50} 0.25 µM). Whilst, no significant inhibition was obtained in the presence of HoK3 in which the α-NH_2 histidyl residues were acetylated, suggesting that interactions between ODN and histidylated oligolysines were involved. Prêt-Luc cells, seeded onto 24-well plates (2 $X10^5$ cells/well), were treated for

4 h at 37°C in DMEM supplemented with 2 % FBS containing various concentrations of GEM-91, (■) in the absence of histidylated oligolysine, (•) in the presence of 20 µM HoK2 or (o) in the presence of 20 µM HoK3. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. Then, FBS was raised to 6 % and cells were further incubated for 18 h. Luciferase gene expression was measured by recording luminescence for 4 s. The percentage of luciferase inhibition was calculated by using , $[(RLU^{ODN}-RLU)/RLU]$ X 100 where RLU^{ODN} and RLU were the luciferase activity into cell lysates of cells incubated in the absence and in the presence of ODN, respectively. Results shown typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation.--

Replace the paragraph beginning at page 31, line 14, with the following rewritten paragraph:

--Figure 2 shows the inhibitory effect of TNF-α induced ICAM-1 expression by ISIS 1939 (CCCCCACCACTTCCCCTCT) (SEQ ID NO:2), an antisense phosphorothioate oligonucleotide (PS-ODN) targeted to the 3' non-coding region of ICAM-1 mRNA. The results showed that TNF-α induced ICAM-1 expression was inhibited by ISIS 1939 in the presence of 20 µM of histidylated oligolysines. HoK2 (IC_{50} of 0.25 µM) appeared to be more efficient than HoK1 (IC_{50} of 0.5 µM) probably because HoK2 bore less histidyl residues than HoK1 (15 *versus* 12). The inhibition was very low in the absence of histidylated oligolysines even up to 1 µM ODN (20 %

inhibition). A549 cells (ATCC CCL 185, Rockville, MD) were plated onto 96-wells microtiter plates (10^4 cells/well). The day after, culture medium was removed and cells were washed. Cells were incubated at 37°C for 4 h in 100 µl DMEM serum-free medium containing ISIS 1939 ODN either in the absence (■) or in the presence of 20 µM (●) HoK1 or (□) HoK2. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. One volume of fresh medium containing 10 ng/ml TNF-α was added and cells were further incubated for 18 h. ICAM-1 expression was quantified by ELISA using anti-ICAM-1 antibodies. Cells were washed 3 times with 200 µl of PBS and fixed for 20 min at room temperature in PBS containing 20 mg/ml paraformaldehyde. Then, cells were incubated for 90 min at 37°C with anti-ICAM 1 mouse antibody (Becton Dickinson) diluted 20 times in PBS containing 20 mg/ml BSA. Cells were washed 3 times with PBS and then incubated for 1 h at 37°C with an anti-mouse horseradish peroxydase conjugate (Becton Dickinson) diluted 2000 times in PBS containing 20 mg/ml BSA. After 3 washes, the peroxydase activity was assessed by using 100 µl of o-phenylenediamine dihydrochloride peroxydase substrate tablet set (Sigma). After X min incubation at 37°C, the reaction was stopped by adding 25 µl of 3 N H_2SO_4 and the absorbance read at 492 nm. All calculations were made relative to untreated controls in the absence or in the presence of TNF-α. The percentage of TNF-α-induced expression of ICAM-1 was calculated as follows : $[A_{TNF-\alpha}{}^{ODN} - A_0) / (A_{TNF-\alpha}-A_0)] X$

100 where $A_{TNF-\alpha}{}^{ODN}$ was the absorbance of ODN treated and cytokine-induced cells, A_0 the absorbance of cells incubated without ODN and TNF-α, and $A_{TNF-\alpha}$ the absorbance of cytokine-induced cells incubated without ODN. Results shown are typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation of the percentage of control ICAM-1 expression induced by TNF-α.--

Replace the heading appearing at page 38, line 2, with the following rewritten heading:

--Example 7 : Preparation of (SEQ ID NO:7) (K(His)-KL(His)-L)$_7$--.

Replace the paragraph beginning at page 38, line 3, with the following rewritten paragraph:

--An oligomer (K(His)-KL(His)-L)$_7$ (SEQ ID NO:8) can be entirely synthesised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer (K(His)-KL(His)-L)$_7$ is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The polymer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.--

Replace the heading appearing at page 38, line 12, with the following rewritten heading:

--Example 8 : Preparation of (SEQ ID NO:9) (K(His)-L-K(His))$_7$--.

Replace the paragraph beginning at page 38, line 13, with the following rewritten paragraph:

--A oligomer (K(His)-L-K(His))$_7$ (SEQ ID NO:9) can be entirely synthesised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The oligomer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.--

REMARKS

The specification has been amended to conform to the accompanying Sequence Listing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Respectfully submitted,

YOUNG & THOMPSON

By _____

Andrew J. Patch
Attorney for Applicants
Registration No. 32,925
745 South 23rd Street
Arlington, VA 22202
Telephone: 521-2297

October 9, 2001

Version with markings to show changes made

IN THE SPECIFICATION:

Paragraph beginning at line 10 and ending at line 20 of page 21 has been amended as follows:

As example of oligonucleotides, one may cite the following:

GEM 91

phosphorothioate (X = S) oligonucleotide i = 25 (SEQ ID NO: 1)

CTC TCG CAC CCA TCT CTC TCC TTC T

complementary to the AUG initiation site of gag HIV-1 gene

ISIS 1939

phosphorothioate (X = S) oligonucleotide i = 19 (SEQ ID NO: 2)

CCC CCA CCA CTT CCC CTC T

complementary to the 3' non coding region of ICAM-1 mRNA.--

Paragraph beginning at line 25 of page 25 has been amended as follows:

As an example of the specific TFO is an oligonucleotide 5'-$A_4GA_4G_6A$-3' (SEQ ID NO: 6) directed against the polypurine track (PPT) in the NEF-HIV-1 gene.

Paragraph beginning at line 1 of page 27 has been amended as follows:

A) Cells are incubated for 4 h at 37°C with 1 µM fluorescein-labelled peptide (*F*-*S*-CGEEDTSEKDEL) (SEQ ID NO:3) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.

Paragraph beginning at line 10 of page 27 has been amended as follows:

B) Dendritic cells are incubated for 4 h at 37°C with 1 µM c-myc epitope peptide (SMEQKLISEEDLNFELDEA) (SEQ ID NO:4) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde in the presence of 0.5 % saponine, washed and then incubated for 1 h with anti c-myc epitope monoclonal antibody (9E10) in PBS containing [containing] 10 mg/ml BSA and 0.1% saponin. Cells are washed and further incubated for 1 h in the presence of fluorescein-labelled anti-mouse IgG F(ab)' fragments in PBS containing [containing] 10 mg/ml BSA and 0.1% saponin. Cells are washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent.

Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.

Paragraph beginning at line 9 of page 28 has been amended as follows:

Dentritic cells were incubated for 4 h at 37°C with the nonadecapeptide (185-203) from the C-terminal part of the HIV-1 Nef protein containing the nonapeptide (190-198) (AFHHVAREL) (SEQ ID NO:5) in the absence or in the presence of an histidylated oligolysine. Cells are washed and further incubated for 24 h at 37°C in the absence of peptide and histidylated oligolysine. MHC class I presentation of peptide antigen was evaluated by Cr^{51} cytoxic assay by using a CTL clone sensible to the peptide. DCs were labeled with Cr^{51} (target cells : T) and then incubated at 37°C for 4 h in the presence of the CTL clone (effector cells : E) at E/T ratios ranged from 1 to 100. The supernatants are collected and the radioactivity in the supernatant was recorded. The % of specific Cr^{51} release is calculated according to $(A_{NS} - A_S)/A_{NS} \times 100$ where A_{NS} and A_S are the radioactivity in supernatant dilutions of dentritic cells incubated in the absence and the presence of CTL cells, respectively.

Paragraph beginning at line 20 of page 30 has been amended as follows:

Figure 1 shows the activity of GEM-91, an antisense phosphorothioate oligonucleotide (PS-ODN) (CTC TCG CAC CCA TCT

CTC TCC TTC T) (SEQ ID NO:1) complementary to the AUG initiation site of gag HIV-1 gene. The effect of histidylated oligolysines was evaluated by using pRET-Luc cells (a rabbit smooth muscle cell line). These cells produce endogenous luciferase under the control of the human phosphoglycerate kinase promoter and the luciferase gene sequence around the AUG codon was replaced by the initiator AUG codon and several downstream codons of gagHIV-1 gene. The results showed that the activity of GEM-91 (IC_{50} > 5 µM) was increased more than 10 times in the presence of 20 µm HoK2 (IC_{50} 0.25 µM). Whilst, no significant inhibition was obtained in the presence of HoK3 in which the α-NH_2 histidyl residues were acetylated, suggesting that interactions between ODN and histidylated oligolysines were involved. Prêt-Luc cells, seeded onto 24-well plates (2 $X10^5$ cells/well), were treated for 4 h at 37°C in DMEM supplemented with 2 % FBS containing various concentrations of GEM-91, (■) in the absence of histidylated oligolysine, (●) in the presence of 20 µM HoK2 or (o) in the presence of 20 µM HoK3. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. Then, FBS was raised to 6 % and cells were further incubated for 18 h. Luciferase gene expression was measured by recording luminescence for 4 s. The percentage of luciferase inhibition was calculated by using , [$(RLU^{ODN}-RLU)/RLU$] X 100 where RLU^{ODN} and RLU were the luciferase activity into cell lysates of cells incubated in the absence and in the presence of ODN, respectively. Results shown

typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation.

Paragraph beginning at line 14 of page 31 has been amended as follows:

Figure 2 shows the inhibitory effect of TNF-α induced ICAM-1 expression by ISIS 1939 (CCCCCACCACTTCCCCTCT) (SEQ ID NO:2), an antisense phosphorothioate oligonucleotide (PS-ODN) targeted to the 3′ non-coding region of ICAM-1 mRNA. The results showed that TNF-α induced ICAM-1 expression was inhibited by ISIS 1939 in the presence of 20 µM of histidylated oligolysines. HoK2 (IC_{50} of 0.25 µM) appeared to be more efficient than HoK1 (IC_{50} of 0.5 µM) probably because HoK2 bore less histidyl residues than HoK1 (15 *versus* 12). The inhibition was very low in the absence of histidylated oligolysines even up to 1 µM ODN (20 % inhibition). A549 cells (ATCC CCL 185, Rockville, MD) were plated onto 96-wells microtiter plates (10^4 cells/well). The day after, culture medium was removed and cells were washed. Cells were incubated at 37°C for 4 h in 100 µl DMEM serum-free medium containing ISIS 1939 ODN either in the absence (■) or in the presence of 20 µM (•) HoK1 or (□) HoK2. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. One volume of fresh medium containing 10 ng/ml TNF-α was added and cells were further incubated for 18 h. ICAM-1 expression was quantified by ELISA using anti-ICAM-1 antibodies. Cells were washed 3 times with 200 µl of PBS and fixed for 20 min

at room temperature in PBS containing 20 mg/ml paraformaldehyde. Then, cells were incubated for 90 min at 37°C with anti-ICAM 1 mouse antibody (Becton Dickinson) diluted 20 times in PBS containing 20 mg/ml BSA. Cells were washed 3 times with PBS and then incubated for 1 h at 37°C with an anti-mouse horseradish peroxydase conjugate (Becton Dickinson) diluted 2000 times in PBS containing 20 mg/ml BSA. After 3 [whashes] washes, the peroxydase activity was assessed by using 100 µl of o-phenylenediamine dihydrochloride peroxydase substrate tablet set (Sigma). After X min incubation at 37°C, the reaction was stopped by adding 25 µl of 3 N H_2SO_4 and the absorbance read at 492 nm. All calculations were made relative to untreated controls in the absence or in the presence of TNF-α. The percentage of TNF-α-induced expression of ICAM-1 was calculated as follows : $[A_{TNF-\alpha}{}^{ODN} - A_0) / (A_{TNF-\alpha}-A_0)] \times 100$ where $A_{TNF-\alpha}{}^{ODN}$ was the absorbance of ODN treated and cytokine-induced cells, A_0 the absorbance of cells incubated without ODN and TNF-α, and $A_{TNF-\alpha}$ the absorbance of cytokine-induced cells incubated without ODN. Results shown are typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation of the percentage of control ICAM-1 expression induced by TNF-α.

Heading appearing at line 2 of page 38 has been amended as follows:

Example 7 : Preparation of (SEQ ID NO:7) (K(His)-KL(His)-L)$_7$.

Paragraph beginning at line 3 of page 38 has been amended as follows:

An oligomer (K(His)-KL(His)-L)$_7$ (SEQ ID NO:8) can be entirely synthesised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer (K(His)-KL(His)-L)$_7$ is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The polymer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.

Heading appearing at line 12 of page 38 has been amended as follows:

Example 8 : Preparation of (SEQ ID NO:9) (K(His)-L-K(His))$_7$.

Paragraph beginning at line 13 of page 38 has been amended as follows:

A oligomer (K(His)-L-K(His))$_7$ (SEQ ID NO:9) can be entirely synthesised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with

conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The oligomer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.

SEQUENCE LISTING

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PICHON, CHANTAL
BELLO-ROUFAI, MAHAJOUB
MONSIGNY, MICHEL

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<141> 2001-06-04

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<151> 1999-11-22

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(Document title)

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(Document title)

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